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Reply to : Oncolytic Viral Therapy for Malignant Pleural Mesothelioma

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Running title: *BAP1* status and the type I interferon pathway in mesothelioma

Key words: Oncolytic immunotherapy, measles virus, type I interferon, gene homozygous deletion, mesothelioma.

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Among the most frequent genomic alterations in malignant pleural mesothelioma (MPM) are the homozygous deletions (HD) of the *CDKN2A* tumor suppressor gene. These alterations are found in 49% of MPM patients according to a recent analysis of TCGA, The Cancer Genome Atlas [1]. In our recent study published in the Journal of Thoracic Oncology performed on 78 short-term–cultured MPM cell lines, we found *CDKN2A* HD in 73% of patients [2]. We also showed that the *CDKN2A* HD are accompanied in 30% of them by the HD of all genes encoding type I interferons (IFN I) that are essential for cellular antiviral defense. Finally, we identified two MPM cell lines with a constitutively activated IFN I response characterized by the expression of numerous IFN-stimulated genes (ISG) that resist to the oncolytic activity of the vaccine Schwarz strain of Measles virus (MV).

Other frequent genomic alterations in MPM are the mutations and/or the HD of the *BAP1* gene [1]. In their letter to the editor, Yang H *et al* focus on The Cancer Genome Atlas (TCGA) data and show that *BAP1* mutation and *BAP1* HD are found in 22% and 14% of patients respectively. They performed a functional enrichment analysis using Gene Ontology and Reactome databases to identify the signal pathways that correlate to *BAP1* expression. They show a strong negative correlation between *BAP1* expression and a constitutively activated IFN I response. They conclude that *BAP1* loss-of-function may be used as a marker of tumors with constitutively activated IFN I response and thus resistance to oncolytic virotherapy.

Hmeljak J et al previously performed an analysis of TCGA on 74 MPM patients with a detailed focus on *BAP1* alteration and its consequences [1]. They report an IFN I signature in MPM tumors with an inactivated *BAP1* gene (Supplementary Figure 3H and Supplementary Figure 7D). Thus, the expression of *BAP1,* as well as its mutational status suggest a negative regulation of the IFN I pathway in MPM by BAP1 protein.

Considering those results, we first analyzed *BAP1* gene expression in 19 MPM cells lines for which we previously determined their MV-sensitivity [3]. We observed a significant enrichment of low *BAP1*-expressing MPM cell lines that are MV-resistant (p=0.02) (**Figure 1A**). We then screened *BAP1* gene alterations in these cell lines. Eight MPM cell lines harbor *BAP1* alterations, 3 with HD and 5 with mutations (**Figure 1B**). These data show that all MPM cell lines with *IFNB1* deletion are sensitive to MV independently of the *BAP1* mutation status. Interestingly, a slight enrichment of *BAP1* genetic alterations was observed in MV-resistant compared to MV-sensitive MPM cell lines (p=0.07), especially considering MPM cell lines without *IFNB1* deletion (p=0.06) (**Figure 1C**).

We thus analyzed the IFN I signature of 36 short-term–cultured MPM cell lines for which *IFNB1* and *BAP1* genetic alteration status have been determined (61% with *BAP1*, 28% with *INFB1*, 14% with both genes alteration). We performed a pathway enrichment analysis using transcriptomic data previously generated from these cell lines [4] and the online pathway analytic tool of Reactome Knowledgebase (https://reactome.org/). We did not find any significant enrichment of IFN I pathways on genes whose expression inversely correlates with *BAP1* gene expression (Spearman´s $r < -0.4$, FDR-adjusted $p < 0.01$), neither on differentially expressed genes between MPM cell lines with or without *BAP1* genetic alteration (data not shown). This absence of link between *BAP1* expression or alteration and IFN I pathways was also observed in our previous study [2]. In absence of virus, MV-resistant Meso61 cell line with a bi-allelic deletion and low expression of *BAP1* harbors an inactivated IFN I response, while MV-resistant Meso45 with a high expression of wild-type *BAP1* harbors a strong constitutively activated IFN I.

Altogether, our results suggest a correlation between *BAP1* gene expression or alteration status and the resistance to MV, but we cannot conclude on a link with the IFN I response. This discrepancy of the results between tumor samples and cell lines may be explained by the microenvironment. It was reported that *BAP1*-altered MPM tumors show a different pattern of infiltration by immune cells than wild-type *BAP1* tumors [5] and these infiltrations may play a role in the observed IFN I signature. Furthermore, this IFN I signature may be responsible for the presence of infiltrated immune cells as it was reported for other cancers [6].

In conclusion, the negative correlation of *BAP1* expression and the IFN I signature in MPM observed by Yang H et al could have a great interest in the context of absence of *INFB1* deletion to identify patient with a lower chance to respond to oncolytic virotherapy using IFN I-sensitive oncolytic viruses. It would thus be interesting to determine the mechanism of the IFN I response modulation by the BAP1 protein.

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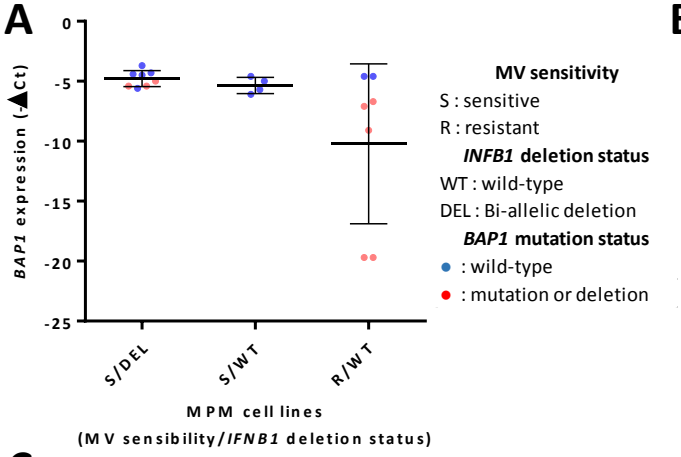
Figure legends

Figure 1: Comparison of *BAP1* gene expression and genetic alteration status to Measles virus sensitivity in malignant pleural mesothelioma cell lines. *BAP1* gene expression and mutation status were analyzed in 19 malignant pleural mesothelioma (MPM) cell lines characterized for Measles virus (MV) sensitivity in our previous study [3]. **A.** *BAP1* gene expression was determined by RT-qPCR as previously described [7]. MV resistant cell lines showed a significantly lower *BAP1* expression than MV sensitive cell lines (Mann Whitney test, p-value=0.02). **B.** *BAP1* mutation status was defined using a targeted next generation sequencing [8]. Biallelic deletions were detected based on sequencing coverage. Sequencing data analysis showed mutations in 8 cell lines: (i) 3 cell lines with bi-allelic deletions of *BAP1* exons, (ii) 2 with nonsense mutations, (iii) 2 with splice site mutations and (iv) 1 with missense mutation predicted to be functionally damaging by polyphen HDIV and SIFT. C. MV sensitivity and *INFB1* and *BAP1* mutations profile in MPM cell lines were resumed in a heat map. *BAP1* mutation and deletion are enriched in MV resistant cell lines compared to MV sensitive cell lines (Fisher's exact test, p=0.07 in all MPM cell lines and p=0.06 in MPM cell lines without *INFB1* deletion). Statistical tests were performed using GraphPad Prism version 6.07 software.

Figure 1

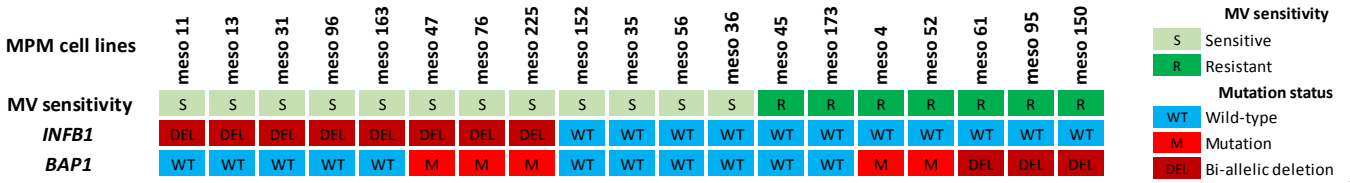
A



B

Sample name	Mutation type	Genomic position/ deleted exons	Protein change
meso 4	nonsense	chr3:52439233T>A	K337*
meso 47	splice site	chr3:52443569C>T	
meso 52	splice site	chr3:52443857C>A	
meso 61	deletion	exon 6	
meso 76	missense	chr3:52441264T>G	H169P
meso 95	deletion	all exons	
meso 150	deletion	exons 14-17	
meso 225	nonsense	chr3:52436624G>A	Q684*

C



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